



**UNIVERSITI PUTRA MALAYSIA**

**CALLUS FORMATION, SOMATIC EMBRYOGENESIS AND  
DEVELOPMENT OF TRANSFORMATION PROTOCOL BY PARTICLE  
BOMBARDMENT IN PLANTAIN BANANA**

**PUSPITA DESWINA**

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BOMBARDMENT IN PLANTAIN BANANA**

**By**

**PUSPITA DESWINA**

**Thesis Submitted in Fulfilment of the Requirement for the Degree of Master  
of Science in the Faculty of Science and Environmental Studies**

**Universiti Putra Malaysia**

**December 2001**

## Dedicated To:

*My Parents: Papa Yuzar Akmam and Mama Djawanis*

*My beloved husband: Heroriki  
My dearest son: Ibnu Khalil Ibram  
for their constant love during my study*

*Brothers and Sister: Dodi Indra (Uda Dodi); Media Sandra Kasih (Uni Med);  
Jecky Aulia(Tet)*

Abstract of thesis presented to senate of Universiti Putra Malaysia in fulfilment  
of requirement for the degree of Master of Science

**CALLUS FORMATION, SOMATIC EMBRYOGENESIS AND  
DEVELOPMENT OF TRANSFORMATION PROTOCOL BY  
PARTICLE BOMBARDMENT IN PLANTAIN BANANA**

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**December 2001**

**Chairman : Professor Hj. Marziah Mahmood, Ph.D.**

**Faculty : Science and Environmental Studies**

The plantain bananas are among the most valued crop plants in the tropical world. However, the commercially attainable yields are very low compared to dessert bananas. Conventional breeding of this plant remains a difficult endeavor because of high sterility and polyploidy; therefore, the integration of biotechnology into plantain improvement programmes is essential. Somatic embryogenesis has the great potential for rapid and efficient regeneration of plantlets and offers opportunities for large-scale production of plant material. The yield and quality of somatic embryos produced in cell culture depends on the media constituents and explants type. In this study, *in vitro* rhizomes initiated from shoot tips (8 week-old cultures) of cultivars Nangka and Tanduk and immature male flowers of cultivar Nangka were used as explants. The results of callus initiation showed that treatments with 2,4-D, Picloram and Dicamba produced callus ranging from 0.0–82.0% for cultivar

Nangka and 0.0–58.0% for cultivar Tanduk. The type of callus varied depending on concentrations of PGRs used for the initiation. However, no callus formation was obtained from the auxin-free medium. The study on the determination of callus growth curve showed that treatments with 2,4-D and Picloram for both cultivars Nangka and Tanduk produced a sigmoidal pattern, except for several concentrations which failed to show the callus growth pattern. Whereas, for the Dicamba treatments, there was no callus growth at all. The effects of two basal media supplemented with 2,4-D and Picloram at different concentrations were studied. The highest callus fresh weight (0.37 g) was attained on SH medium plus 2.5  $\mu$ M Picloram. The effect of 2,4-D and Picloram in combination with other auxins and cytokinins, showed that there was no constant trend on callus growth in response to the treatments. However, the treatments using 2,4-D and Picloram in combination with other cytokinins produced nodular and compact callus. The callus produced embryogenic structure (13.3–66.7%) in the somatic embryogenesis medium, but no shoot regeneration was achieved, instead only roots were formed. In the initiation of callus from immature male flowers, 49.7% of flower cluster responded to form globular embryogenic callus and the highest response was from flower of rows 12 and 13 (60%). The study on the development of transformation protocol by particle bombardment using *in vitro* rhizome slices and embryogenic callus as explants and GFP fluorescent gene as the reporter gene revealed an unstable transient expression in the cell.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master of Sains

**PEMBENTUKAN KALUS, EMBRIOGENESIS SOMA DAN  
PENGHASILAN PROTOKOL TRANSFORMASI MELALUI  
PEMBEDILAN ZARAH DI DALAM PISANG PLANTAIN**

**Oleh**

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Pisang plantain adalah diantara kebanyakan tanaman yang bernilai tinggi di kawasan tropika. Walau bagaimanapun, hasil keluaran komersil yang diperolehi daripadanya sangat rendah berbanding dengan pisang pencuci mulut. Pembiakan biasa tanaman ini masih sukar disebabkan oleh kadar kesterilan dan poliploidi yang tinggi. Oleh itu, integrasi bioteknologi dalam program memperbaiki pisang plantain memerlukan protokol kultur sel yang sesuai, di mana embriogenesis soma mempunyai potensi yang tinggi untuk pembiakan dalam jumlah besar, cepat dan cekap. Hasil dan kualiti daripada embrio soma yang dihasilkan dalam kultur sel bergantung kepada kandungan media dan eksplan berbeza yang digunakan dalam proses pengkulturan tersebut. Dalam kajian ini, dua jenis eksplan telah diguna iaitu rizom *in vitro* daripada kultivar Nangka dan Tanduk serta bunga jantan yang belum matang daripada kultivar Nangka. Keputusan yang diperolehi daripada inisiasi kalus menunjukkan rawatan dengan 2,4-D, Picloram dan Dicamba menghasilkan pembentukan

kalus antara 0.0–82.0% daripada kultivar Nangka dan 0.0–58.0% untuk kultivar Tanduk. Jenis kalus berbeza-beza bergantung kepada kepekatan pengawalatur tumbesaran yang digunakan untuk inisiasi. Walau bagaimanapun, tidak terdapat pembentukan kalus daripada media tanpa auksin. Kajian keatas penentuan pertumbuhan kalus dengan rawatan 2,4-D dan Picloram untuk kultivar Nangka dan Tanduk telah dapat menghasilkan lengkung pertumbuhan sigmoid, kecuali bagi beberapa kepekatan. Manakala rawatan dengan Dicamba, tidak menghasilkan pertumbuhan kalus. Kesan daripada dua media dasar yang dibekalkan dengan 2,4-D dan Picloram pada kepekatan yang berbeza menghasilkan gumpalan kalus yang banyak dalam media SH ditambah dengan 2.5  $\mu$ M Picloram. Kesan 2,4-D dan Picloram dengan gabungan auksin dan sitokinin yang lain menunjukkan tidak terdapat kecenderungan yang tetap diperolehi diantara rawatan. Walau bagaimanapun, pada rawatan 2,4-D dan Picloram yang digabung dengan sitokinin yang lain boleh menghasilkan kalus yang bernodul dan padat, kalus ini bertukar kepada struktur embriogenik (13.3 – 66.7%) di dalam media embriogenesis soma, tetapi tidak ada regenerasi pucuk yang diperolehi. Walau bagaimanapun tindakbalas embriogenik hanya membentuk akar. Sebaliknya, inisiasi kalus daripada bunga jantan yang belum matang hanya menghasilkan kalus embriogenik bernodul ( $\pm$ 49.7%) dan kelompok yang berpotensi membentuk kalus bernodul adalah pada baris 12 dan 13 (60%). Kajian terhadap pembangunan protokol transformasi melalui pembedilan zarah menggunakan potongan rizom *in vitro* dan kalus embriogenik sebagai eksplan, dan GFP gen berpendaflor sebagai gen penanda didapati pengutaraan transien ke dalam sel tidak stabil.

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May ALLAH bless us always.....



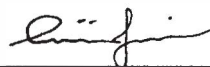
I certify that an Examination Committee met on 7<sup>th</sup> December 2001 to conduct the final examination of Puspita Deswina on her Master of Science thesis entitled “Callus Formation, Somatic Embryogenesis and Development of Transformation Protocol by Particle Bombardment in Plantain Banana” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science.



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I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



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PUSPITA DESWINA

Date : **2** JAN 2002

## TABLE OF CONTENTS

	<b>Page</b>
<b>DEDICATION</b>	ii
<b>ABSTRACT</b>	iii
<b>ABSTRAK</b>	v
<b>ACKNOWLEDGEMENTS</b>	vii
<b>APPROVAL SHEETS</b>	viii
<b>DECLARATION FORM</b>	x
<b>LIST OF TABLES</b>	xiv
<b>LIST OF FIGURES</b>	xv
<b>LIST OF PLATES</b>	xvii
<b>LIST OF ABBREVIATIONS</b>	xix
 <b>CHAPTER</b>	
 <b>1 INTRODUCTION</b>	 1
1.1 Importance of Plantains	1
1.2 Improvement of Plantains and Bananas through Biotechnology	4
1.3 Objectives	6
 <b>2 LITERATURE REVIEW</b>	 7
2.1 The Plantains	7
2.2 <i>In vitro</i> Plant Regeneration System	9
2.2.1 Auxins	10
2.2.2 Cytokinins	11
2.3 <i>In vitro</i> Regeneration of Plantain and Banana	13
2.4 Callus Induction	15
2.5 Somatic Embryogenesis	17
2.6 Plant Transformation Strategies	21
2.6.1 <i>Agrobacterium</i> -mediated Transformation	22
2.6.2 Microprojectile Bombardment	22
2.6.3 Reporter Genes	24
2.6.4 Green Fluorescent Protein Genetic Marker	26
 <b>3 CALLUS FORMATION, OPTIMIZATION AND SOMATIC EMBRYOGENESIS FROM <i>IN VITRO</i> RHIZOMES AND IMMATURE MALE FLOWERS OF CULTIVARS NANGKA AND TANDUK</b>	 28
3.1 Introduction	28
3.2 Materials and Methods	30
3.2.1 Shoot tip Explant Preparation and Culture	30
3.2.2 Induction of Callus from <i>In vitro</i> Rhizome	32

3.2.2.1	Preparation of <i>In vitro</i> Rhizome	32
3.2.2.2	Initiation of Callus	32
3.2.3	Optimization of Callus Production and Somatic Embryogenesis	33
3.2.3.1	Determination of Callus Growth Curves	33
3.2.3.2	Assessment of Basal Media for Optimization of Callus Production	33
3.2.3.3	Effect of 2,4-D and Picloram in Combination with other Auxins on Callus Production and Embryogenic Callus Formation	34
3.2.3.4	Effect of 2,4-D and Picloram in Combination with other Cytokinins on Callus Production and Embryogenic Callus Formation	34
3.2.3.5	Optimization of Somatic Embryogenesis	35
3.2.3.6	Regeneration and Germination of Globular Embryos	35
3.2.4	Preparation and Culture of Immature Male Flowers	36
3.2.4.1	Preparation of Medium and Culture of Immature Male Flowers	36
3.3	Results and Discussions	38
3.3.1	Callus Induction from <i>In vitro</i> Rhizome	38
3.3.2	Determination of Callus Growth Curves	50
3.3.3	Assessment of Basal Media for Optimization of Callus Production	51
3.3.4	Effect of 2,4-D and Picloram in Combination with other Auxins on Callus Growth and Embryogenic Callus Formation	57
3.3.5	Effect of 2,4-D and Picloram in Combination with other Cytokinins on Callus Growth and Embryogenic Callus Formation	61
3.3.6	Induction and Regeneration of Somatic Embryos	66
3.3.7	Callus Induction and Formation of Somatic Embryos from Immature Male Flowers	74
4	<b>PRELIMINARY DEVELOPMENT OF TRANSFORMATION PROTOCOL BY PARTICLE BOMBARDMENT IN PLANTAIN BANANA</b>	
4.1	Introduction	79
4.2	Materials and Methods	80
4.2.1	Preparation of Plasmid	80
4.2.2	Plasmid Mini-prep of pGEM Ubi 1-5 gfp S65T	81
4.2.3	DNA Quality and Quantitation	81
4.2.4	Agarose Gel Electrophoresis	82
4.2.5	Preparation of Explants	82
4.2.6	Transformation Procedure	83

4.2.6.1 DNA- gold Particle Precipitation	83
4.2.6.2 Bombardment Conditions	83
4.2.6.3 Post Bombardment Tissue Handling	84
4.3 Results and Discussion	84
4.3.1 Percentages of GFP Spots	84
4.3.2 Numbers and Stability of GFP spots	86
<b>5 CONCLUSION</b>	<b>90</b>
<b>REFERENCES</b>	<b>94</b>
<b>APPENDICES</b>	<b>108</b>
<b>BIODATA OF THE AUTHOR</b>	<b>116</b>

## LIST OF TABLES

Table	Page
3.1 Response of <i>in vitro</i> rhizomes of cultivar Nangka on different concentrations of 2,4-D, Picloram and Dicamba.	39
3.2 Response of <i>in vitro</i> rhizomes of cultivar Tanduk on different concentrations of 2,4-D, Picloram and Dicamba.	40
3.3 Response of callus derived from <i>in vitro</i> rhizome on somatic embryogenesis medium.	67
3.4 Response of embryogenic callus derived from <i>in vitro</i> rhizome of cultivar Nangka on regeneration medium.	71
3.5 Response of immature male flower explants of cultivar Nangka during the culture period.	75
3.6 Percentage of immature male flower clusters forming nodular callus according to their position.	76
4.1 Transformation efficiency of GFP spots on embryogenic callus culture and rhizome slices of cultivar Nangka using different pressure and target distance.	85

## LIST OF FIGURES

Figures	Page
3.1 Effect of different concentrations of 2,4-D, Picloram and Dicamba in MS medium on callus fresh weight (g) in (a) cultivar Nangka and (b) cultivar Tanduk.	42
3.2 Growth of callus on different concentrations of 2,4-D in (a) cultivar Nangka and (b) cultivar Tanduk.	52
3.3 Growth of callus on different concentrations of Picloram in (a) cultivar Nangka and (b) cultivar Tanduk.	53
3.4 Growth of callus on different concentrations of Dicamba in (a) cultivar Nangka and (b) cultivar Tanduk.	54
3.5 Effect of two basal media supplemented with different concentrations of Picloram on callus production of cultivar Nangka. (a) MS medium, (b) SH medium.	55
3.6 Effect of two basal media supplemented with different concentrations of 2,4-D on callus production of cultivar Nangka. (a) MS medium, (b) SH medium.	56
3.7 Effect of 2,4-D and Picloram in combination with other auxins on callus growth of cultivar Nangka. (a) 1 $\mu$ M 2,4-D + different auxins (0.5 $\mu$ M) (b) 4 $\mu$ M Picloram + different auxins (0.5 $\mu$ M)	58
3.8 Effect of 2,4-D and Picloram in combination with different concentration of (a) Picloram and (b) 2,4-D on callus growth of cultivar Nangka.	60
3.9 Effect of 2,4-D and Picloram in combination with other cytokinins on callus growth of cultivar Nangka. (a) 1 $\mu$ M 2,4-D + different cytokinins (0.5 $\mu$ M), (b) 4 $\mu$ M Picloram + different cytokinins (0.5 $\mu$ M).	62



3.10	Effect of 2,4-D and Picloram in combination with different concentrations of (a) BAP and (b) Zeatin on callus growth of cultivar Nangka.	63
4.1	The numbers of GFP spots detected after 48 h in callus at (a) 1350 psi, (b) 1100 psi and rhizome slices at (c) 1350 psi, (d) 1100 psi with different target distance.	87

## LIST OF PLATES

Plate	Page
1.1 Musa cultivars (a) Nangka (b) Tanduk	3
3.1 Preparation of <i>in vitro</i> rhizomes for callus induction from shoot tip cultures of cultivar Nangka. (a) Shoot tip with 3-4 leaf primordial after 8 weeks of culture, (b) sample of <i>in vitro</i> rhizome.	31
3.2 Preparation of immature male flowers for callus initiation (a) in cultivar Nangka, with the bracts removed until the inflorescence size reached $\pm 5$ cm (b), $\pm 1$ cm (c) and (d) flower clusters ranging from # (1–15).	37
3.3 Morphology of callus on different concentrations of 2,4-D. (a) Control with shoot formation, (b) nodular, compact and yellowish-white callus on 1.0 $\mu$ M, (c) nodular, compact and yellowish-white callus on 2.0 $\mu$ M, (d) nodular, compact and yellowish-white callus on 4.0 $\mu$ M, (e) small and yellowish on 6.0 $\mu$ M (f) small and yellowish on 8.0 $\mu$ M (g) small and watery callus on 10.0 $\mu$ M of cultivar Nangka	44
3.4 Morphology of callus on different concentrations of Picloram (a) Control with shoot formation, (b) vitreous callus with shoot on 1.0 $\mu$ M, (c) vitreous callus with shoot on 2.0 $\mu$ M, (d) friable and green callus on 4.0 $\mu$ M, (e) friable and green callus on 6.0 $\mu$ M, (f) small nodular structure and whitish callus on 8.0 $\mu$ M, (g) small and watery callus on 10.0 $\mu$ M of cultivar Nangka	45
3.5 Morphology of callus on different concentrations of Dicamba. (a) Control with shoot formation, (b) vitreous callus with shoot on 1.0 $\mu$ M, (c) vitreous callus and whitish on 2.0 $\mu$ M, (d) friable and yellowish callus on 4.0 $\mu$ M, (e) nodular and yellowish callus on 6.0 $\mu$ M (f) watery and yellowish callus on 8.0 $\mu$ M (g) vitreous and whitish callus on 10.0 $\mu$ M of cultivar Nangka.	46

3.6	Morphology of callus on different concentrations of 2,4-D. (a) Control with shoot formation, (b) small, nodular and yellowish callus on 1.0 $\mu$ M, (c) small, nodular and yellowish callus on 2.0 $\mu$ M, (d) small, nodular and yellowish callus on 4.0 $\mu$ M, (e) small, nodular and yellowish callus on 6.0 $\mu$ M, (f) vitreous and whitish callus on 8.0 $\mu$ M (g) vitreous and whitish callus on 10.0 $\mu$ M of cultivar Tanduk.	47
3.7	Morphology of callus on different concentrations of Picloram (a) Control with shoot formation, (b) browning explant on 1.0 $\mu$ M, (c) swelling and green callus on 2.0 $\mu$ M, (d) nodular, compact and greenish callus on 4.0 $\mu$ M, (e) nodular, compact and greenish callus on 6.0 $\mu$ M (f) nodular, compact and greenish callus on 8.0 $\mu$ M (g) nodular, compact and greenish callus on 10.0 $\mu$ M of cultivar Tanduk.	48
3.8	Morphology of callus on different concentrations of Dicamba (a) control with shoot formation, (b) vitreous callus with shoot on 1.0 $\mu$ M, (c) vitreous callus on 2.0 $\mu$ M, (d) vitreous and whitish callus on 4.0 $\mu$ M, (e) vitreous and whitish callus on 6.0 $\mu$ M (f) vitreous and whitish callus on 8.0 $\mu$ M (g) vitreous and whitish callus on 10.0 $\mu$ M of cultivar Tanduk.	49
3.9	Nodular and compact callus on (a) 1 $\mu$ M 2,4-D + 4 $\mu$ M BAP and (b) embryogenic callus on 1 $\mu$ M 2,4-D + 10 $\mu$ M BAP.	65
3.10	Microscopic observation of cells derived from globular embryos of cultivar Nangka (stained with Iodine Solution). (a) embryogenic cells with starch granules and (b) non-embryogenic cells which are large and empty.	68
3.11	Root formation from the globular embryos structure of cultivar Nangka on 40 $\mu$ M BAP + 0.5 $\mu$ M Zeatin.	72
3.12	Induction of embryogenic callus from immature male flower of cultivar Nangka after 3 week of cultures (a) and globular embryogenic callus from cluster # 12, after 20 week of cultures (b).	77
4.1	Transient expression of GFP gene detected 48 h after bombardment at 6 cm target distance and 1100 psi helium pressure in (a) embryogenic callus and (b) rhizome slice. of cultivar Nangka.	89

## LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celcius
2,4-D	2,4-Dichlorophenoxyacetic acid
BAP	6-benzylaminopurine
br	Browning
cm	Centimetre
conc.	Concentration
ctrl	Control
cul.	Culture
d	Day
dH <sub>2</sub> O	Distilled water
Dicamba or Dic	3,6-Dichloro-o-aniscic acid
EDTA	Ethylenediaminetetraacetic acid (ferric sodium salt)
e.g.	Example
EtOH	Ethanol
FW	Fresh weight
g	Gram
h	Hours
i.e.	That is
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
Kinetin or Kin	6-furfurylaminopurine
L	Litre
mg	Milligram
mm	millimeter
min	Minute
mL	Millilitre
mM	Millimolar
Na <sub>2</sub> EDTA	EDTA disodium salt
NAA	α-Naphtaleneacetic acid
NaOH	Sodium hydroxide
NPT II	Neomycin phosphotransferase
nr	no response
nm	nanometre
PGR(s)	Plant growth regulator(s)
Picloram or Pic	4-Amino-3,5,6-trichloropicolinic acid
SDS	Sodium dodecyl sulphate
Zeatin	6-(4-Hydroxy-3-methyl-but-2-enylamino) purine
v/v	Volume for volume
w/v	Weight for volume
N	Normality
μ	Specific growth rate (d)
μl	Microlitre
μg	Microgram
μM	Micromolar

## CHAPTER I

### INTRODUCTION

#### 1.1. Importance of Plantains

Plantains are important staple food crops for certain groups of people living in the tropics and they are among the cheapest sources of starch (Swennen, 1990; Rowe, 1998). According to Dadzie (1995) plantain banana has practically the same nutritional value as dessert banana, but the carbohydrate content mainly consists of starch rather than sugar. In general, plantain and banana provides a good source of carbohydrates, minerals such as potassium, magnesium, phosphorous, calcium, iron and also vitamins A and C (Horry, 1990; Kodym and Zapata-Arias, 1999). Latham (1979) reported that banana starch is easily digested and is thus suitable for the preparation of food for infants. On the other hand, the nature of the carbohydrates varies widely between cultivars and within a particular cultivar during different stages of ripening (Vuylsteke *et al.*, 1990).

Plantains consisted of one-half of the total world output of bananas (Swennen, 1990). Nevertheless, several countries make no distinction in their statistics between banana and plantain production and publish only the overall estimates (FAO, 1998). To date, very little progress has been made in the improvement of plantain banana; therefore, it is important for agriculturists to

develop a systematic research program in improving plantain banana as a food crop.

In this study, two plantain cultivars (Nangka and Tanduk) which are commercially popular in Malaysia (Jamaluddin, 1990) were investigated.

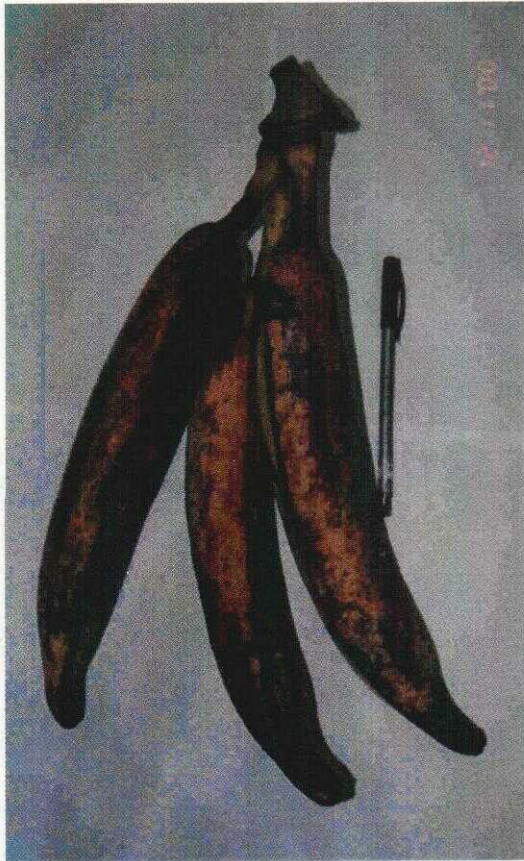
- Cultivar Nangka (AAB)

The fruit skin is thick and remains light green in color when ripe. The flesh is creamy white, fine textured, starchy and subacid in taste. The fruit ranges from 18 to 24 cm in length, 3.5 to 5.0 cm wide and has good keeping quality. The large hands have 14 to 24 fingers and the fruit bunch has 6 - 8 hands (Kusumo and Dasi, 1989) (Plate 1.1a).

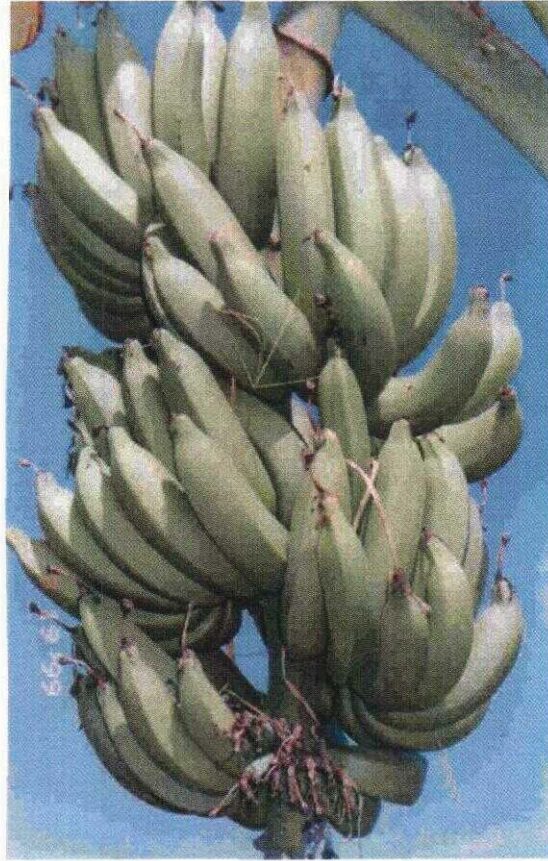
- Cultivar Tanduk (AAB)

It is popular known as "horn plantain", and is also the largest fruit among the bananas. It has limited commercial value in Malaysia due to its poor yield. The fruit has excellent keeping quality and remains starchy even when fully ripe, and requires cooking to become palatable. The fruit ranges from 25.0 to 35.0 cm in length and from 5.0 - 6.5 cm in diameter. The bunch weight is only 7 - 10 kg. The skin is yellow in colour when ripe and the pulp is





(a)



(b)

Plate 1.1 : *Musa* cultivars (a) Tanduk (b) Nangka

light colour with fine but firm texture. Generally, there are two hands in a bunch and sometimes only one hand. There are no male flower buds formed for this cultivar ( Swennen, 1990) (Plate 1.1b).

## **1.2. Improvement of Plantains and Bananas through Biotechnology**

Plantain and banana production is hampered by several diseases such as black Sigatoka , *Fusarium* wilt and banana bunchy top virus (BBTV) disease, which resulted in an increased effort to genetically improve the crop (Vuylsteke *et al.*, 1993; Lee *et al.*, 1997). Robinson (1996) reported two major problems in all plantain cultivars which are highly susceptible to the black Sigatoka fungus, and commercially low attainable yields compared to dessert bananas. Conventional breeding of *Musa* spp. poses problems such as high sterility and polyploidy nature of most of the edible cultivars (Vuylsteke *et al.*, 1993; May *et al.*, 1995; Schoofs *et al.*, 1997). Therefore, biotechnological approaches such as through mutation breeding, somaclonal variation or genetic engineering may have great potential in the genetic enhancement of plantains and bananas. Hence, some scientists advocated these biotechnological approaches as the solution to genetically improve the crop.

Crouch *et al.* (1998) stated that genetic modification of *Musa* spp. offers the opportunity to add desirable gene characteristics into target tissue by genetic transformation technique. The establishment of a transformation protocol is necessary which includes the preparation of a target material for the



transformation and the regeneration of transformed plant. One of the target materials used in the transformation of *Musa* spp. is somatic embryo. Somatic embryos have proven to be the ideal materials as plantlets produced are non-chimeric and the multiplication is rapid (Panis and Swennen, 1993). The potential use of somatic embryogenesis for rapid and efficient regeneration of plantlets is practically essential in genetic transformation work (Crouch *et al.*, 1998 and JayaSree *et al.*, 2001). Moreover, somatic embryogenesis is useful in agriculture because it fixes the genotype to that of the female parent (Grapin *et al.*, 2000).

The genetic improvement of this crop through the transfer of foreign genes into target cells have been achieved using embryogenic cell suspension (Panis and Swennen, 1993; Sagi *et al.*, 1995; *et al.*, 2001), protoplasts cell ( Sagi *et al.*, 1994; 1995 ) and rhizome slices ( May *et al.*, 1995) by *Agrobacterium*-mediated transformation and particle bombardment. However, the regeneration of whole plants from cultured somatic cells is still a major limiting step in the application of genetic engineering for the improvement of banana crop (Novak, 1992; Crouch *et al.*, 1998; Kodym & Zapata-Arias, 1999) and success is also genotype-specific (Rout *et al.*, 2000).

This project is divided into two parts, the induction of callus and somatic embryogenesis, and the development of transformation protocol in cultivars Nangka and Tanduk.